IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Lightner *et al.*
Application No. 10/539,215
Filed: January 17, 2006
Confirmation No. 5991
For: GENERATION OF PLANTS WITH ALTERED OIL CONTENT
Examiner: Elizabeth F. McElwain
Art Unit: 1638
Attorney Reference No. 7896-71314-07

SUBMITTED VIA EFS

COMMISSIONER FOR PATENTS
SUBMITTED VIA ELECTRONIC FILING SYSTEM

DECLARATION UNDER 37 C.F.R. §1.132

1. I, Dr. John P. Davies, hold the position of Senior Director, Discovery Research, at Exelixis Plant Sciences, Portland, Oregon. I have a Ph.D. in Molecular Biology and have 24 years of experience working in the field of plant physiology. My *curriculum vitae* is attached as **Exhibit A**. I have supervised the grow-out experiments described herein and I was involved in the analysis of the gene expression experiments described herein, which are an extension of the work described in the above-referenced application (U.S. Patent Application No. 10/539,215; hereinafter the '215 application).

2. Over-expression of the *Arabidopsis* gene At3g58750, encoding citrate synthase (SEQ ID NO: 1; GenBank entry GI# 30694870) in *Arabidopsis* correlates with an increase in seed oil content. Using methods as described in Example 1 of the '215 application, the *Arabidopsis* gene At3g58750 (encoding citrate synthase) was cloned behind the strong constitutive CsVMV promoter in a binary expression vector and transformed into *Arabidopsis* using Agrobacterium-mediated transformation. T1 seed harvested from the primary transformant was spread on medium containing a selective agent and transformed T1 seedlings identified. The transgenic seedlings were grown to maturity and T2 seed harvested. Seed from each T1 plant is considered to contain a separate transgenic event and designated as a family. Twenty-two transgenic T2 seedlings from a single family and 10 non-transformed control

seedlings (Col-0) were planted individually into cells of a 32-cell tray. The plants were allowed to grow to maturity, self-pollinate and set seed. Each tray contained transgenic plants from a T2 family designated by the sample name. Oil content from transgenic plants was compared with the control seed grown in the same tray. After seed harvest, seed oil content was determined by Near Infrared Spectroscopy as described in the specification. Of the ten T2 sample families tested, four families (samples ZX00850001, ZX00850002, ZX00850004 and ZX00850011) showed an increase in seed oil content (t-test P value < 0.05; **Exhibit B**).

3. *Arabidopsis* plants transformed with the CsVMV::At3g58750 transgene (encoding citrate synthase) as described in Paragraph 2 of this Declaration over-express At3g58750 transcript compared to wild-type plants. T2 seed from sample families ZX00850001, ZX00850002, ZX00850004, ZX00850007, ZX00850008 and ZX00850009, was planted, germinated and the plants grown for two weeks. The seedlings were harvested and total RNA was isolated. Expression of the At3g58750 in transformed and untransformed control plants was determined using the Taqman assay and compared with expression of At3g58750 in wild-type plants. Transcripts for At3g58750 were more abundant in five of the six T2 sample families tested (ZX00850001, ZX00850002, ZX00850004, ZX00850007 and ZX00850008) compared with wild-type control plants (**Exhibit C**). The one sample family that did not show an increase in *Arabidopsis* citrate synthase gene transcript exhibited no change in expression of this gene transcript. None of the six sample families tested exhibited **any** suppression of *Arabidopsis* gene At3g58750 transcript levels. Therefore, co-suppression of the *Arabidopsis* citrate synthase gene transcript cannot explain increased seed oil content in plants comprising the *Arabidopsis* gene CsVMV::At3g58750 expression vector.

4. Three of the sample families described in Paragraphs 2 and 3 of this Declaration (ZX00850001, ZX0085002 and ZX0085004) demonstrated both increased seed oil content (**Exhibit B**) and increased *Arabidopsis* citrate synthase gene expression (**Exhibit C**). One sample family, ZX00850008, was not tested for oil content and another sample family, ZX00850007, showed average increased oil content (~5%) but the t-test P value was not significant at the 95% confidence interval (P value = 0.055; **Exhibit B**). Since the citrate synthase gene transcript is more abundant in sample families that accumulate increased seed oil

content, increased *Arabidopsis* citrate synthase gene expression correlates with increased seed oil content.

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

John P. Davies, Ph.D.

February 4, 2009
Date

Exhibit A (3 pages)

John P. Davies, Ph.D
Curriculum Vitae

Present Position:

Senior Director, Plant Trait Discovery, Exelixis Plant Sciences, 16160 SW Upper Boones Ferry Road, Portland OR 97224, Phone: 503-213-2165, email: jdavies@exelixis.co

Education and Research Experience:

Senior Director, Plant Trait Discovery, Exelixis Plant Sciences 16160 SW Upper Boones Ferry Rd, Portland OR 97224 (September 2007-present).

Director II, Plant Trait Discovery, Exelixis Plant Sciences 16160 SW Upper Boones Ferry Rd, Portland OR 97224 (September 2003-2007).

Director, Plant Trait Discovery, Exelixis Plant Sciences, 16160 SW Upper Boones Ferry Rd, Portland OR 97224 (September 2001-2003).

Associate Director, Plant Biotechnology, Exelixis, Inc., PO Box 511, 170 Harbor Way South San Francisco CA 94083 (September 2000-September 2001).

Assistant Professor, Department of Botany, Iowa State University, 354 Bessey Hall, Ames, IA 50011 (February 1998-August 2000).

Postdoctoral Associate (April 1990-January 1998) Carnegie Institution of Washington, Department of Plant Biology, 260 Panama St., Stanford, CA 94305. Advisor: Dr. Arthur R. Grossman

Post-doctoral Associate with Dr. Donald P. Weeks (February 1989-March 1990) Sandoz Crop Protection, 975 California Ave, Palo Alto, CA 94304.

Ph.D. 1989. Department of Molecular Biology, Vanderbilt University, Nashville TN 37235. Dissertation: Torsional stress and negative supercoiling in plastid DNA decrease during chloroplast development. Advisor: Dr. Gisela Mosig.

M.S. 1984. Department of Agronomy and Soils, Auburn University, Auburn AL 36830.
Thesis: The effect of tall fescue genotypes of varying root morphology on the growth of white clover. Advisor: Dr. Jeffrey Pedersen.

B.S. 1981, Department of Biology, University of the South, Sewanee, Tennessee 37375.
Major: Biology.

Grants and Awards:

NSF Grant MCB-9975765 Chlamydomonas Genome Project. 1999-2002. Co-PI $289,609 of $3,200,000 total (PI, Dr. A. Grossman; Co-PIs, Dr P. Lefebvre, Univ. MN, Dr. C Silflow, Univ. MN; Dr. D. Stern, Boyce Thompson Research Institute, Dr. E. Harris, Duke Univ., Dr. J. Davies, Iowa State Univ.)

USDA Grant #9900622, Sulfur Stress Signal Transduction. 1999-2002. $200,000.

USDA Grant #96351003142, Acclimation of *Chlamydomonas* to sulfur limitation: Regulation and Survival, 1996-1999. Written with Dr. A. R. Grossman.

USDA Grant #9302076, Acclimation of *Chlamydomonas* to sulfur limitation, 1993-1996. Written with Dr. A. R. Grossman.

USDA Grant #9103011, Transformation of *C. reinhardtii* to examine sulfur-regulated gene expression, 1991-1993. Written with Dr. A. R. Grossman.

Service Committees

-USDA Plant Responses to the Environment Grant Review Panel, 2001.

-Member of the Steering Committee of the Chlamydomonas Genome Project 1998-2000.

-Co-Chair "Chlamydomonas Genome Project" section at the 1998 meeting on The Cell and Molecular Biology of Chlamydomonas.

Publications:

1. Walsh TA, Bauer T, Neal R, Merlo AO, Schmitzer PR, Hicks GR, Honma M, Matsumura W, Wolff K, Davies JP. (2007) Chemical genetic identification of glutamine phosphoribosylpyrophosphate amidotransferase as the target for a novel bleaching herbicide in Arabidopsis, Plant Physiol. 144:1292-304.
2. Walsh TA, Neal R, Merlo AO, Honma M, Hicks GR, Wolff K, Matsumura W, Davies JP (2006) Mutations in an Auxin Receptor Homolog AFB5 and in SGT1b Confer Resistance to Synthetic Picolinate Auxins and not to 2,4-D or IAA in Arabidopsis, Plant Physiol. 142:542-52.
3. Kimura, T, Shibagaki, N., Ohkama-Ohtsu, N., Hayashi, H., Yoneyama, T., Davies, J.P., and Fujiwara, T. (2006) Arabidopsis SNRK2.3 protein kinase is involved in the regulation of sulfur-responsive gene expression and O-acetyl-l-serine accumulation under limited sulfur supply. Soil Science and Plant Nutrition 2006 **52**:211.
4. Shrager, J., Hauser, C., Chang, C-W, Harris, E.H., Davies, J.P., McDermott, J.P., Tams, R., Zhang, Z., and Grossman, A.R., (2002) *Chlamydomoans reinhardtii* Genome Project: A guide to the generation and use of the cDNA information. Plant Physiol. 131:401-8.
5. Ravina, C.G., Chang, C-I., Tsakraklides, G.P., McDermott , J.P., Vega, J.M., Leustek, T., Gotor, C., and Davies, J.P. (2002) The sac mutants of Chlamydomonas reinhardtii reveal transcriptional and post-transcriptional control of cysteine biosynthesis. Plant Physiol. 130:2076-84.
6. Shibagaki, N, McDermott, J. and Davies, J.P. (2002) Selenate-resistant mutants of *Arabidopsis thaliana* identify *Sultr1;2*, a sulfate transporter required for efficient transport of sulfate into roots. Plant Journal 29: 475-486.
7. Shrager J, Chang, C-W, Davies J, Harris EH, Hauser C, Tamse R, Surzycki R, Gurjal M, Zhang Z, Grossman AR. (2001) Chlamydomonas cDNAs; Assembly and potential role in understanding metabolic processes. In: Proceedings of the 12th International Congress on Photosynthesis. Brisbane, Australia.
8. Leustek, T., Martin, M.N, Bick, J-A., and Davies, J.P. (2000) Pathways and Regulation of Sulfur Metabolism Revealed Through Molecular Genetic Studies. Ann. Rev. Plant Physiol. Plant Mol. Biol. 51: 141-166.

9. Davies, J.P., Yildiz, F.H., and Grossman, A. (1999) *Sac3*, an Snf1-like serine/threonine kinase that positively and negatively regulates the responses of *Chlamydomonas reinhardtii* to sulfur limitation. Plant Cell 11:1179-1190.
10. Davies, J.P., and Grossman, A.R. (1999) The use of Chlamydomonas as a model algal system for genome studies. J. Phycol. 34:907-917.
11. Wykoff, D.D., Davies, J.P., Melis, A. and Grossman, A.R. (1998) The regulation of photosynthetic electron transport during nutrient deprivation of *Chlamydomonas reinhardtii*. Plant Physiol. 117:129-139.
12. Davies, J.P., and Grossman, A.R. (1998) Survival during macronutrient limitation, in *Molecular Biology of Chloroplasts and Mitochondria of Chlamydomonas* (Rochaix, J.-D., Goldschmidt-Clermont, M., and Merchant, S. eds.) pp 613-635.
13. Yildiz, F.H., Davies, J.P., and Grossman, A.R., (1996) Sulfur availability and the SAC1 gene control adenosine triphosphate sulfurylase gene expression in *Chlamydomonas reinhardtii*, Plant Physiol. 112: 669-675.
14. Davies, J.P., Yildiz, F.H. and Grossman (1996) Sac1, a putative regulator that is critical for survival of *Chlamydomonas reinhardtii* during sulfur deprivation, EMBO J 15:2150-2159.
15. Davies, J.P. and Grossman, A.R. (1994) Sequences controlling transcription of the *Chlamydomonas reinhardtii* β_2-tubulin gene after deflagellation and during the cell cycle, Molecular and Cellular Biology 14:5165-5174.
16. Yildiz, F.H., Davies, J.P., and Grossman, A.R., (1994) Characterization of sulfate transport in *Chlamydomonas reinhardtii* during sulfur-limited and sulfur-sufficient growth, Plant Physiology, 104:981-987.
17. Davies, J.P., Yildiz, F., and Grossman A. R. (1994) Generation and characterization of mutants affecting sulfur-regulated gene expression in *Chlamydomonas reinhardtii*. The Plant Cell 6:53-63.
18. Davies, J.P., D.P. Weeks and A.R. Grossman (1992). Expression or the arylsulfatase gene from the β_2-tubulin promoter in *Chlamydomonas reinhardtii*. Nucleic Acids Research, 20:2959-2965.
19. Davies, J. P., Thompson, R. J., and Mosig, G. (1991) Intercalation of psoralen into DNA of plastid chromosomes decreases late during barley chloroplast development, Nucleic Acids Res. 19: 5219-5225.
20. Thompson, R. J., Davies, J. P., Lin, G., and Mosig, G. (1990) Modulation of transcription by altered torsional stress, upstream silencers, and DNA-binding proteins, in *The Bacterial Chromosome* (Drilica, K. and Riley, M. eds.) American Society for Microbiology, Washington, D.C. 20005.
21. Thompson, R. J., Davies, J. P., and Mosig, G. (1985) 'Dark lethality' of certain *Chlamydomonas reinhardtii* strains is prevented by dim blue light. Plant Physiol. 79:903-907.

Exhibit B (1 page)

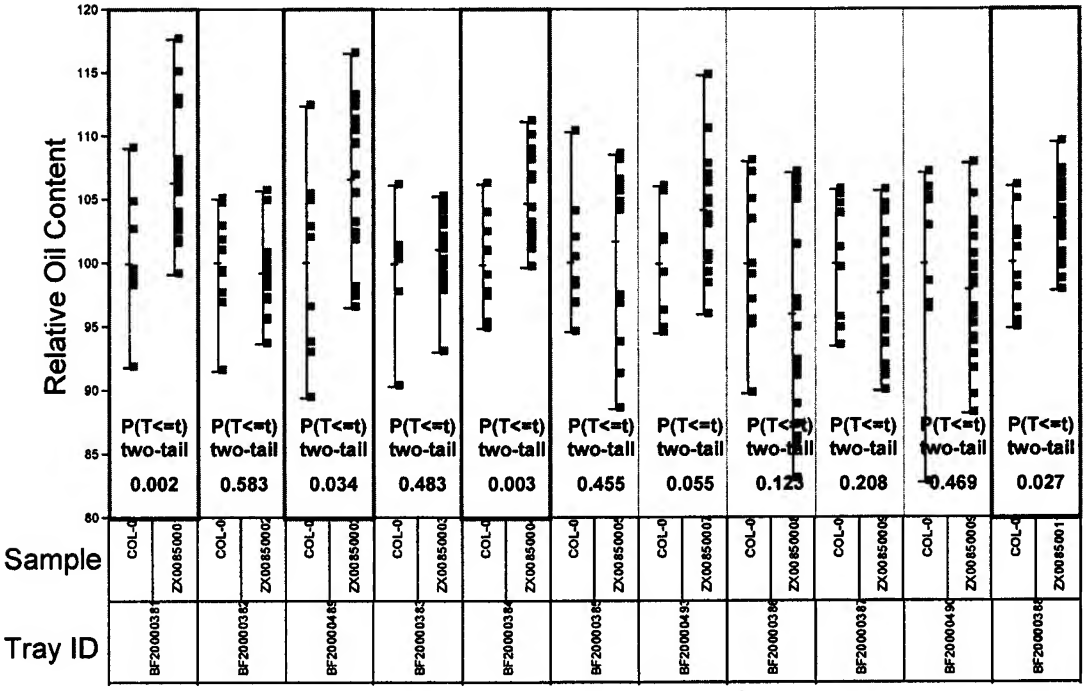

Exhibit C (1 page)